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The University of Birmingham
Edgbaston
Birmingham
B15 2TT, England

Patents ADP number *(if you know it)* 7736890064

If the applicant is a corporate body, give the country/state of its incorporation — England

4. Title of the invention — FLUID-FLOW CONTROL DEVICE

5. Name of your agent *(if you have one)* — Marks & Clerk

"Address for Service" in the United Kingdom to which all correspondence should be sent *(including the postcode)*

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Description 29

Claim(s)

Abstract

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FLUID-FLOW CONTROL DEVICE

The present invention relates in a first aspect to a fluid-flow control device.

There are a large number of assay devices for determining chemical or biochemical analytes in a sample. One common type of device comprises a strip of porous material impregnated in at least one discrete region with a reagent sensitive to the analyte of interest. The reagent is usually chosen so that the presence of the analyte is readily observable (eg. appearance or change in colour or fluorescence). A sample is applied to one region of the strip and liquid (eg. water) is applied to the same or another region. As an alternative, the sample may be the liquid or contained in the liquid. The liquid is drawn by capillary action along the strip and carries the sample through the region(s) containing the reagent(s), until saturation of the strip occurs when liquid reaches the far end of the strip. Any analyte of interest present in the sample will contact the reagent(s) as the liquid passes along the strip. Such devices may include built-in procedural controls and/or end of test indicators which are well known in the art.

One disadvantage of such devices is that the contact period between sample and reagent is dependent upon the rate of flow of the sample across the region(s) containing the reagent(s). For certain analyte/reagent pairs, this period may not be long enough for sufficient reaction to occur, i.e. such a device lacks the necessary sensitivity to measure the presence of certain analytes.

US 5874216 discloses a device having a chromatographic strip and a separate sample preparation region on opposable components which can be brought into opposition. The sample preparation region may incorporate a reagent with which the analyte of interest binds and after an appropriate reaction time the opposable components are brought together so that the sample contacts the chromatographic strip and flows into a detection zone.

A successful assay may be dependent upon the user allowing the correct time to elapse between sample preparation and application of sample to the chromatographic strip.

US 4727019 discloses a device which partly overcomes the above disadvantage. The device comprises a porous membrane in contact with an absorbent member. The porous membrane is impregnated with a receptor for a ligand of interest. Sample containing the ligand of interest is applied to the upper side of the membrane and is drawn through the membrane into the absorbent member by capillary action. A labelled receptor which also binds the ligand of interest is then applied to the porous membrane to react with the receptor-ligand complex so that the receptor-ligand complex may be observed. Sensitivity of the reaction may be controlled by applying a large amount of sample to the membrane and/or allowing a longer time period between applying the sample and the labelled receptor.

However, disadvantages are (i) the use of such a device may require large amounts of sample, (ii) the sample and labelled ligand are applied to the

membrane manually and (iii) the period between application of the sample and ligand must be measured.

None of the prior art devices are able to control the assay by controlling the flow of the liquid along the porous medium.

Thus, an object of the present invention is to provide a fluid-flow control device capable of use as an assay device eg. for determining chemical or biochemical analytes present in a sample. Another object is to provide a device which obviates or mitigates one or more disadvantages of the prior art assay devices.

According to a first aspect of the present invention, there is provided a fluid-flow control device comprising:-

(i) a fluid flow path,

(ii) a fluid flow barrier at a location in the flow path, and

(iii) barrier release means in the flow path which, in use, is moveable by fluid flowing in the flow path into contact with the flow barrier so as to permit fluid flow through said location.

It will be understood that there is no flow through said location until the barrier is contacted in use by the barrier release means.

Preferably, said fluid is a liquid and more preferably an aqueous liquid.

Preferably, the fluid flow barrier is a non-wetting region in the flow path and the release means is a wetting agent capable of wetting said non-wetting region, so as to enable flow of liquid through said region.

Preferably, the fluid flow barrier is a hydrophobic material and the release means is a surfactant. Examples of suitable hydrophobic materials include waxes (e.g. paraffin wax), resins (e.g. acrylic resin based varnish, 100% acrylic polymer varnish, coumarone-indene resin) and commercially available inks and paints. Examples of suitable surfactants include octyl-β-D-glucopyranoside (ODG), dioctyl sulfosuccinate sodium salt (DOSS), polyoxyethylene (23) dodecyl ether (BRIJ 35) and polyoxyethylene (20) sorbitan monolaurate (TWEEN 20).

Preferably, the flow path is defined by interstices (i.e. capillaries and/or pores) of a porous medium. More preferably said porous medium is a nitrocellulose-based material.

Preferably, the fluid flow barrier is impregnated into and immobilised on the porous medium. Preferably, the barrier release means is impregnated into the porous medium and is soluble in the flowing fluid.

Preferably, the device is constructed so that, in use, there is at least one region in the flow path where the direction of fluid flow is different, after fluid flow through said location is initiated compared with the direction of fluid flow in that region before fluid flow through said location is initiated. More preferably the fluid flow direction is reversed.

The present invention also resides in an assay device for an analyte comprising

(i) a flow path for a liquid defined by interstices in a porous medium,

(ii) at least one liquid flow barrier which is impregnated into and immobilised on the porous medium at a location in the flow path,

(iii) at least one barrier release means which is impregnated into the porous medium, which is soluble in the liquid, and which, in use, is moveable by liquid flowing in the flow path into contact with the flow barrier so as to permit liquid flow through said location, and

(iv) an analyte capture region in the flow path.

Preferably, an analyte visualising agent is provided at a zone in the flow path, said visualising agent being capable of interacting with the analyte to indicate the presence of the analyte. Alternatively, the device may be provided with a visualising agent application zone adapted to receive a visualising agent.

As used herein, the expression "visualising agent" encompasses any agent which interacts with the analyte directly or indirectly via an intermediary analyte binding reagent (eg. enzyme labelled conjugate) and which enables the visual or instrumental detection of analyte qualitatively, semi-quantitatively or quantitatively eg. by colour, fluorescence, luminescence or radioactivity. It will be understood that the visualising agent may be inherently detectable, or detectable once interaction with the analyte or intermediary analyte binding reagent has occurred.

In a first embodiment, the visualising agent is immobilised in the capture region and also serves to immobilise the analyte in the capture region.

In a second embodiment, the analyte capture region includes an immobilised analyte binding substance which serves to immobilise analyte in the capture region and the visualising agent is upstream of the capture region so that in use, it flows through the capture region.

The device may include a sample application zone in the flow path which may, if present, correspond to the analyte capture region or the visualising agent application zone. The sample application zone may be surrounded by an additional flow barrier and an associated barrier release means. Alternatively, the sample to be analysed may be included in the liquid which flows, in use, along the flow path.

In a third embodiment, at least two barriers and at least two barrier release means are provided and arranged so that, in use, some liquid flows over the analyte capture region at least twice.

In those embodiments where more than one barrier is present, the barriers may have different constitutions to each other. Similarly where more than one barrier release means is present, the barrier release means may have different constitutions to each other.

Embodiments of the invention will now be described by way of Example only, with reference to the accompanying drawings, in which:-

Figure 1 show a flow control device in accordance with the present invention used to test various barriers and surfactants,
Figures 2a and 2b show assay devices in accordance with the present invention for incubating a sample and reagent at a capture site (Figure 2a), or for incubating a sample and reagent before flowing over a capture site (Figure 2b),
Figure 3 shows an assay device in accordance with the present invention for causing repeated sample flow across a capture site,
Figure 4 shows an assay device in accordance with the present invention for first delivering a large sample volume through a capture site, followed by analyte labelling,
Figure 5 shows an assay device in accordance with the present invention for isolating a sample region from its surroundings,
Figure 6a shows a membrane strip for use in an assay device in accordance with the present invention where a relatively large volume of sample moves vertically through the thickness of the strip and horizontally along the strip, and
Figure 6b is a sectional view of the assay device incorporating the strip of Figure 6a.

In the following Examples, the use of "up", down", "left" and "right" and other reference directions relates to the orientations shown in the drawings.

Example 1 Porous media, barrier and surfactant interaction

Referring to Figure 1 a polyester supported nitrocellulose membrane sheet of 3 µm or 8 µm nominal pore size (Whatman International Ltd,

Maidstone England) was cut into a 10 mm wide by 60 mm long strip 11 with a barrier 12, 20 mm from one end 13. The barrier 12 was applied to the sheet using a Type MP5200 flat bed plotter (Graphtec Corporation, Yokohama, Japan) prior to cutting. A short perpendicular line 16 was drawn manually at each end of the barrier 12 along the cut edges of the strip 11 using a Lumocolor pen. The strip 11 was dried for 10 minutes at 35° C in an air circulating oven before use. Several such strips 11 were prepared from each sheet and strips were stored in sealed containers prior to use.

In use, the end 13 of the strip was dipped vertically into about a 3 mm depth of liquid (water or surfactant-containing water, the surfactant serving as a barrier release means), in a vessel 14. Liquid wicked up the membrane to the barrier 12 and the time taken for the liquid to penetrate ("switch") the barrier 12 was noted. The pen lines 16 prevented liquid circumventing the barrier and wicking up the cut edges of the strip 11. To see the passage of liquid more clearly a blue line 15 was manually drawn just below the barrier using a water soluble 0.6 mm "Note Writer" pen (Berol, Banford, Norfolk,UK).

The flow characteristics of different combinations of membrane, barrier and surfactant were investigated. Tables 1 to 11 show for each combination (i) the maximum permissible concentration (%) of surfactant in the liquid (water) to delay barrier switching by at least ten minutes (max conc. %), (ii) the concentration (%) of surfactant required to allow rapid switching of the barrier (switching conc. %), and (iii) the transition time (switching time) across the barrier for liquid at the same concentration as

(ii), defined as the time in seconds from liquid touching the underside of the barrier to when it starts to appear on the opposite side of the barrier (in the absence of barrier the transition time is <5s).

Table 1 - Yellow Pen Barrier[1] (8 μm membrane)

	ODG[2]	DOSS[3]	BRIJ 35[4]	TWEEN 20[5]
max conc. %	0.03	0.015	0.5	0.5
switching conc. %	0.125	0.06	2.5	5
Transition time (s)	16	14	60	43

[1]Paint Marker Type 751 (Edding AG, Ahrensburg, Germany)
[2]ODG (Octyl-β-D-glucopyranoside) (Fluka, Buchs, Switzerland).
[3]DOSS (Dioctyl sulfosuccinate, sodium salt) (Sigma-Aldrich Co Ltd, Poole, UK).
[4]BRIJ 35 (Polyoxyethylene (23) dodecyl ether) (ICN Biolchemicals, Cleveland, USA).
[5]TWEEN 20 (Polyoxyethylene(20)sorbitan monolaurate) (Sigma-Aldrich Co Ltd.)

Table 2 - Green Pen Barrier[1] (8 μm membrane)

	ODG	DOSS	BRIJ 35	TWEEN 20
max conc. %	0.03	0.015	0.5	0.5
switching conc. %	0.25	0.06	2.5	5
Transition time (s)	16	14	60[2]	43

[1]Paint Marker Type 780 (Edding AG) fitted with a tip from pen type 751.
[2]5% BRIJ 35 has the same transition time but flows more slowly thereafter

Table 3 - White Pen Barrier[1] (8 μm membrane)

	ODG	DOSS
max conc. %	0.125	0.03
switching conc. %	1.25	0.125
Transition time (s)	11	26

[1]Paint Marker Type 751
(5% TWEEN and 5% BRIJ showed slow uneven switching.)

Table 4 - Silver Pen Barrier[1] (8 μm membrane)

	ODG
max conc. %	0.125
switching conc. % Transition time (seconds)	1.25 10

[1]Paint Marker Type 751

(5% TWEEN 20, 5% BRIJ and 1% DOSS did not penetrate the silver barrier.)

Table 5 - Yellow Pen Barrier (3 μm membrane)

	ODG	DOSS	BRIJ 35
max conc. %	0.06	0.03	1.25
switching conc. % Transition time (s)	2.5 9	1.0 45	5 45

(2.5% TWEEN 20 gave slow and uneven switching)

Table 6 - Green Pen Barrier (3 μm membrane)

	ODG	DOSS	BRIJ 35
max conc. %	0.06	Not Done	1.25
switching conc. % Transition time (s)	2.5 11	1.0 60	5 45

(2.5% TWEEN 20 showed slow and uneven switching)

Table 7- White Pen Barrier (3 μm membrane)

	ODG
max conc. %	0.125
switching conc. % Transition time (s)	2.5 12

(5% TWEEN, 5% BRIJ and 1% DOSS showed slow & uneven switching)

Table 8 – Lumocolor[1] diluted 1 in 4 with 1-propanol (8 μm membrane)

	ODG	ODG
max conc. %	0.125	0.125
switching conc. %	2.5	1.25
Transition time (s)	5	60

[1]Staedtler, Nuernberg, Germany

Table 9 - Lumocolor diluted 1 in 2 with 1-propanol (8 μm membrane)

	ODG	ODG
max conc. %	0.125	0.125
switching conc. %	2.5	1.25
Transition time (s)	5	180

Table 10 - Coumarone resin[1] (8 μm membrane)

Barrier line width (mm)	% ODG		% TWEEN 20
	1.25	0.062	0.5
1.4[2]	5	>600	9
3.8[3]	7	>600	24
4.8[4]	13	>600	111
	Transition time (s)		

[1]Prepared by dissolving: 1 g poly coumarone-co-indene resin, Mn 1090 (Sigma-Aldrich) with 4 g dioxane to give a 20% wt/wt stock solution. The working solution was 0.5 ml of stock solution to which was added 3x0.5 ml heptane mixing between each addition (resultant solution: 5.27% resin wt/vol solvents). Barriers were dried at 35°C prior to testing with surfactants.

[2]single line plotted with 0.5 mm pen at 5 cm/s

[3]single line plotted with 0.5 mm pen at 1 cm/s

[4]three lines, 1 mm apart, plotted with 0.5 mm pen at 1 cm/s

Table 11- Wax Barrier (8 µm membrane) - Transition time

% ODG	% Wax[1] in white spirit			
	1.25	2.5	5	10
1.25	~125	~55	~92	~71
2.5	17	~24	11	~12
5	19	20	8	29
10	33	21	19	~76
	Transition time (s)[2]			

[1]Paraffin Wax mp 51-53°C (BDH, Poole UK) solubilised in white spirit (BDH) by heating gently on a hot plate set at 50°C.
[2]Using 8 µm membrane, all wax barriers, from 1.25 to 10% concentration prevented the flow of deionised water for at least 10 minutes (duration of test). Wax barriers below 0.75% wax allow passage of water. 10% TWEEN 20, 10% BRIJ 35, and 1% DOSS did not penetrate the 1.25% or 10% wax.
"~" = Mean time because the transition was not even along the wax line.

Table 12 shows the distance wicked for the wax barrier/ODG surfactant combinations shown in Table 11.

Table 12- Wax Barrier (8 µm membrane)- Distance wicked

% ODG	% Wax			
	1.25	2.5	5	10
1.25	28	36	35	31
2.5	38	49	51[2]	37
5	33	43	47	30
10	26	35	38	26
	Wicking distance (mm) 5 minutes after immersion[1]			

[1]Without a wax barrier the wicking distance of water was 71 mm
[2]5% wax, 2.5% ODG is the optimum combination in respect of rapid switching and distance wicked

Cryla Soluble Gloss Varnish (Daler Rowney, Berkshire, UK) (an acrylic resin based varnish): Using the 8 µm membrane a barrier of 0.1% varnish in white spirit (plotted at 10cm/s using a 0.7 mm pen) held back water for

at least 10 minutes. A line of 2.5% ODG was applied manually (0.7 pen) about 5 mm below the Cryla line and water applied to the end of the strip. The barrier switched in about 12 seconds but flow through the barrier was uneven compared with other barrier materials.

Liquitex High Gloss Varnish (Brinney & Smith Inc., Easton, USA) (100% acrylic polymer varnish): Using the 8 µm membrane a barrier of 80% varnish in water (plotted at 10 cm/s using a 0.7 mm pen) held back water for at least 10 minutes. A line of 2.5% ODG was plotted about 5 mm below the varnish (5 cm/s, 0.7 mm pen) and water applied to the end of the strip. The barrier switched immediately. 70% varnish is marginal as a water barrier because of slight water absorption. This is seen as a bloom on the surface of the varnish after it was applied to an impervious surface, dried, dipped into water for 30 minutes, dried and examined. However, switching was much faster than any of the other barriers and, using an appropriate application method, could be a useful barrier.

Wax and 3µm membrane Wax is difficult to use as a barrier on the 3 µm membrane because wax at a concentration of 0.3% allows water to pass through, but at a concentration of 0.6%, water will not pass through the barrier even at 5% ODG. Thus, the useful working range is very small and the wax content of the barrier critical.

Discussion:

It will be seen that some combinations of porous media, barrier and surfactant result in faster switching of the barrier than others, e.g. 5s for Lumocolor barrier and 2.5% ODG compared with 17s for 1.25% wax

barrier and ODG at the same concentration on the same membrane (Table 8 cf. Table 11). For some combinations, switching occurs at a relatively low concentration of surfactant, e.g. 0.06% DOSS (see Table 1) and some surfactants are ineffective in breaking through some barriers, e.g. TWEEN, BRIJ and DOSS through wax barrier (note 2 to Table 11). From the above data, it is clear that a suitable combination can be chosen for the particular analytical system of interest. For example, if it were necessary to have a first surfactant present as part of the analytical reaction(s) but desirable that this surfactant should not cause switching of the barrier, an appropriate choice may be wax for the barrier and TWEEN 20, BRIJ or DOSS for the first surfactant. switching of the barrier could then be controlled by a second surfactant such as ODG.

The flow control devices of Example 1 do not necessarily reflect the performance of practical assay devices, but are intended to illustrate the difference in behaviour of various barrier/surfactant/membrane combinations. In a preferred assay device the surfactant is dried into an area on the membrane during manufacture. In use, the concentration of surfactant at the barrier is dependent upon the concentration of surfactant applied, its volume, geometry, and the geometry and size of the surrounding structure. Additionally, the switching time is not merely dependent upon the nature of the barrier, surfactant and membrane materials, it is also affected by the degree of saturation in the area of surfactant adjacent to the barrier (saturation is the amount of liquid present per unit area of the membrane). This in turn is dependent upon the geometry and size of the device. This saturation effect can be put to

important use to form a feedback-like mechanism so that, for example, a barrier may open only when the device is substantially saturated.

The following Examples show how the principles described above can be incorporated into practical assay devices for determining, for example, chemical or biochemical analytes present in samples such as water, urine and blood. It will be understood that the specific reagents used will depend upon the specific analyte(s) of interest.

Examples 2a and 2b

Referring to Figure 2a, an assay device comprises a generally rectangular strip of polyester backed 8 μm nitrocellulose membrane 20. Permanent impervious lines 21 formed by the application of neat Lumocolor ink using the pen plotter divide the membrane into first, second and third channels 22,23,24 which extend from the lower end of the device toward its upper end. The second channel 23 is further divided into a lower portion 23a and an upper portion 23b by a horizontal impervious line 25. At their upper end, the first and second channels 22,23 merge into an enlarged common region 24a which separates the upper end of the third channel 24 from the upper ends of the first and second channels 22,23. A switchable barrier 26 (silver paint from pen type 8700 (Edding AG, plotted using a 0.7 mm pen at 1cm/s, and dried at 35° C for 10 minutes) extends across the full width of the first channel 22 and the upper portion 23b of the second channel 23. Spaced above the switchable barrier 26, a line of surfactant 27 (2.5% ODG plotted using a 0.7 pen at 3 cm/s, and dried at 35° C for 10 minutes) extends substantially across the width of the first and second channels 22,23. Spaced below the barrier 26, a capture line

28a comprising an antibody to the analyte of interest immobilised onto the membrane 20, extends substantially across the width of the first channel 22. Spaced below the capture line 28a, also in the first channel 22, is a region 29 containing gold conjugate for labelling the analyte of interest if present in a sample. A solution of methylcellulose is applied to the membrane prior to the addition of the gold conjugate to prevent unspecific binding to the membrane.

In this embodiment, the analyte is one which can form an immunoassay complex such as a protein, eg. for diagnosis of human chorion gonadotropin (hCG), leutinising hormone (hLH) or C-reactive protein (CRP).

In use, the lower end of the device is placed into liquid so that liquid flow ("wicking") is initiated in the first, second and third channels 22,23,24. The liquid may serve as a diluent for a sample to be analysed or it may be the undiluted sample (e.g. urine). In the first channel 22, liquid wicks up to the gold conjugate containing region 29 where the analyte of interest reacts (if present) to form a gold-analyte complex. Liquid flow continues and the gold-analyte complex (as well as any unreacted gold conjugate) is moved by the liquid flow over the capture line 28a until flow is stopped by the barrier 26. The gold-analyte complex is immobilised at the capture line 28a by binding to the antibodies at the capture line 28a. Liquid also flows in the lower portion 23a of the second channel 23, but is prevented from entering the upper portion 23b of the second channel 23 by the impervious line 25 therebetween.

While liquid is stationary in the first channel 22 at the barrier 26 and in the second channel 23 at the line 25, liquid continues to flow into the third channel 24 and down into the common region 24a. The liquid front solubilises the surfactant line 27 and when the liquid reaches the upper side of the switchable barrier 26, the surfactant switches the barrier 26 i.e. converts the capillary structure of barrier 26 from non-wetting to wetting enabling liquid to flow therethrough.

The geometry of the device is such that downward flow from the common region 24a into the first and second channels 22,23 is substantially symmetrical, so that the barrier 26 is switched substantially simultaneously in the first and second channels 22,23. Liquid is rapidly drawn into the dry upper portion 23b of the second channel 23 from the common region 24a which in turn draws liquid from the first channel 22 through the common region 24a into the upper portion 23b of the second channel 23. Hence, any unbound material in channel 22 flows quickly into region 24a, further flow acting to wash the capture line 28a. A small volume of liquid also flows from the third channel 24, through region 24a, into the upper portion 23b of the second channel 23. If the analyte of interest is present in a predetermined amount it will be seen as a coloured line at the capture line 28a. Liquid continues to flow in the first and third channels 22 and 24 until the upper portion 23b of the second channel 23 is saturated or the device is removed from the liquid.

Referring to Figure 2b, a modified assay device is shown which is identical to that shown in Figure 2a except that the capture line 28b is disposed in the first channel 22 between the barrier 26 and the surfactant

line 27. The modified device is particularly useful when the reaction time between analyte and visualising agent (in this embodiment gold-conjugate) is critical, prior to their flow over the capture line 28b.

In use, about 10 µl of sample is applied onto the gold conjugate containing region 29. The device is placed in liquid and liquid flow is as described with reference to the embodiment of Figure 2a. However, since the capture line 28b is above the barrier 26 in this embodiment, the sample and gold conjugate incubate prior to flowing over the capture line 28b. As before, after a predetermined time the barrier 26 switches causing the gold-analyte complex to flow over the capture line 28b. In this embodiment, it is not necessary for methyl cellulose to be applied to the second or third channels 23,24.

EXAMPLE 3

Referring to Figure 3, a polyester backed 8 µm nitrocellulose membrane 30 is divided into channels by impervious pen lines 31 as described for Example 2a. A vertical first channel 32 leads to left and right inner and outer channels 33l,33r;34l,34r at its upper end, its lower end defining the base of the device. Each outer channel 34l,34r extends around the periphery of the membrane 30 and has an enlarged end region 35l,35r remote from the vertical first channel 31. The right outer channel 34r has a convoluted region 36 so that the length of the right outer channel 34r is greater than that of the left outer channel 34l. The left and right inner channels 33l,33r are short in relation to the outer channels 34l,34r and lead into the leftmost and rightmost ends respectively of a horizontal reaction channel 37. The right inner channel 33r also leads into a first

overflow channel 38 which is otherwise closed. The reaction channel 37 is provided with a capture line 39 (containing immobilised antibody to analyte of interest) at its midpoint and a gold conjugate containing region 40 spaced to the left of the capture line 39. In other embodiments, the gold conjugate is replaced by different visualising agents.

The enlarged end regions 35l,35r of the left and right outer channels 34l,34r are separated from second and third overflow regions 41l,41r respectively by a first "Type 1" barrier 42l,42r (prepared by centrifuging the silver paint contained in pen type 8700 at 750 g for 20 minutes, diluting the supernatant therefrom with 5% white spirit and plotting with a 0.5 mm pen at 1 cm/s) and from the left and rightmost ends respectively of the reaction channel 37 by a second similar barrier 43l,43r perpendicular to the first barrier 42l,42r. The first and second barriers 42l,42r;43l,43r were dried at 35°C for 10 minutes. Each enlarged end region 35l,35r contains a line of surfactant 44l,44r (2.5% ODG plotted using a 0.7 pen at 5 cm/s, and dried at 35° C for 10 minutes) spaced from and parallel to the respective first and second barriers 42l,43l;42r,43r.

In use, sample being analysed for an analyte of interest is applied to the gold conjugate containing region 40 and the vertical channel 32 of the device is placed into water (or other liquid) which wicks up the vertical channel 32 and into the left and right inner and outer channels 33l,33r,34l,34r. From the left and right inner channels 33l,33r, water flows into the left and right end respectively of the reaction channel 37. Flow from the left end passes through the gold conjugate containing region 40 and washes gold-analyte complex (and gold-conjugate and

unreacted sample) towards the capture line 39. Since part of the flow from the right inner channel 33r is diverted into the first overflow channel 38, the flow into the right end of the reaction channel 37 is less than into the left end. Thus, there is net flow in the reaction channel 37 from left to right and the sample is carried across the capture line 39 (left to right) for a **first time**, where it reacts with the immobilised antibodies. When the first overflow channel 37 is filled, flow in channel 38 substantially stops.

Simultaneously, water continues to flow along the relatively longer outer channels 34l,34r, but because the left outer channel 34l is shorter than the right outer channel 34r, water arrives at the enlarged end region 35l of the left outer channel 34l before the enlarged end region 35r of the right outer channel 34r. A part-circular water front flows into the surfactant line 44l and water now containing surfactant flows simultaneously to the first and second barriers 42l,43l. The surfactant switches the barriers 42l,43l so that liquid can flow therethrough. Thus, the enlarged end region 35l of the left outer channel 34l is in communication with both the left end of the reaction channel 37 and the second overflow channel 41l. Since the second overflow channel 41l is dry, liquid is drawn into the second overflow channel 41l from the enlarged end region 35l of the left outer channel 34l and the left end of the reaction channel 37. This causes right to left flow in the reaction channel 37 and the sample/gold conjugate passes through the capture line 39 (right to left) for a **second time**.

Meanwhile, water continues to flow in the right outer channel 34r and into its enlarged end region 35r. A part-circular water front flows into the surfactant line 44r and water now containing surfactant flows

simultaneously to the first and second barriers 42r,43r. The surfactant switches the barriers 42r,43r so that liquid can flow therethrough. Thus, the enlarged end region 35r of the right outer channel 34r is in communication with both the right end of the reaction channel 37 and the third overflow channel 41r. Since the third overflow channel 41r is dry, liquid is drawn into the third overflow channel 41r from the enlarged end region 35r of the right outer channel 34r and the right end of the reaction channel 37. This causes left to right flow in the reaction channel 37 and the sample/gold conjugate passes through the capture line 39 (left to right) for a **third time**. This last movement along the reaction channel 37 moves the gold clear of the capture line 39 and provides a wash step so that the capture line 39 can easily be seen.

It will be understood that the distances moved by the sample/gold across the capture line 39 is determined by the volume of the first, second and third overflow regions 38,41l,41r. Such a device offers improved sensitivity when using a small sample volume by allowing sample and reagents to pass several times over the capture line 39.

EXAMPLE 4

Referring to Figure 4, a polyester backed 8 μm nitrocellulose membrane 50 is divided into channels by impervious pen lines of neat Lumocolor ink as described for Example 2a. The membrane 50 has a substantially circular portion 50a and a rectangular base 50b. The periphery of the membrane 50 is marked with an impervious line 51 except for a bottom edge 52 of the base 50b which serves as a liquid inlet. An impervious line 53 extends generally towards the centre of the circular portion 50a

from each side of the base 50b where the base 50b intersects the circular portion 50a to define an open-ended tapering first channel 54. A switchable barrier 55 extends partly across the first channel 54 at the same radius as the impervious line 51 marking the periphery of the membrane 50. A further impervious line 56 extends generally towards the centre of the circular portion 50a from each end of the switchable barrier 55 to define an open-ended tapering second channel 57 wholly within the first channel 54. Within the second channel 57 is disposed a gold conjugate (or other visualising agent) containing region 58 spaced above a line of surfactant 59 (1.25% ODG plotted using a 0.5 pen at 5 cm/s, and dried at 35° C for 10 minutes). A capture line of immobilised antibody 60 is positioned above the first channel 54 and is parallel to and above the switchable barrier 55.

In use, the base 50b of the membrane 50 is placed in a liquid sample. Liquid wicks into the first channel 54 and around the second channel 57, upward flow into the second channel 57 being prevented by the switchable barrier 55. Liquid then flows down into the second channel 57 (moving the gold conjugate and surfactant line towards the switchable barrier 55) and upwards out of the first channel 54 where it forms a part-circular front. The front passes through the capture line 60 and outwards towards the periphery.

The downwardly flowing sample together with the gold conjugate in the second channel 57 wicks into the surfactant line 59 and continues until it contacts the switchable barrier 55. The surfactant acts to convert the capillaries of the barrier 55 from non-wetting to wetting in order for liquid

to flow therethrough. However, flow through the barrier 55 does not occur immediately.

Since the switchable barrier 55 is located at about the same radius as the periphery of the membrane 50, outward flow reaches the periphery at about the same time as downward flow in the second channel 57 reaches the switchable barrier 55. When the saturation at the switchable barrier 55 has reached a critical value (dependent upon the barrier/surfactant combination) the barrier 55 becomes permeable. Outward flow towards the periphery continues drawing liquid through the second (and first) channels 57,54, thereby causing gold conjugate to flow over the capture line 60 until total saturation occurs. Any analyte present in the sample will be visualised at the capture site 60, with any unbound material being washed clear.

Switching of different barriers was investigated using water as the sample and the results were as follows:-

1 A "Type 2" barrier (prepared by diluting 1ml of type 1 barrier material with 250 µl of white spirit and 250 µl of ethanol and plotted using a 0.5 mm pen at 5 cm/s) opened at 3 minutes 50 seconds.

2 White paint barrier plotted using a 0.7 mm pen at 1 cm/s opened at 1 minute 10 seconds (before liquid reached the periphery).

3 1 part Lumocolor in 4 parts 1-propanol barrier plotted using a 0.5 mm pen at 5 cm/s opened at 3 minutes 30 seconds.

This embodiment provides a means of first delivering a large volume of sample through an antibody capture line followed by a visualising agent.

Example 5

Referring to Figure 5, a rectangular polyester backed 8 μm nitrocellulose membrane strip 61 is marked around its periphery by an impervious pen line 62 of neat Lumocolor ink as described for Example 2a except for a short bottom edge 63 which serves as a liquid inlet. Parallel to the bottom edge 63, a first Type 2 material switchable barrier 64 (plotted using a 0.5mm pen at 5 cm/s) extends partly across the strip 61. An impervious line 65 extends perpendicularly from each end of the first barrier 64 away from the bottom edge 63 to define a channel 66 having a closed lower end and an open upper end. Within the channel 66 is a colorimetric reagent containing region 67 spaced above a line of surfactant 68 (2.5% ODG plotted using a 0.7mm pen at 5 cm/s, and dried at 35° C for 10 minutes). Above the channel 66, a capture region 69 is defined within a rectangular line of second Type 2 switchable barrier 70. The capture region 69 contains immobilised antibody and an enzyme conjugate. In this embodiment, the colorimetric reagent is one which interacts with the enzyme conjugate and not the analyte itself. A rectangular line of surfactant 71 (also 2.5% ODG) surrounds the capture region 69 and is spaced from the second barrier 71.

In use, about 3 μl of sample to be analysed for the analyte of interest is applied onto the capture region 69 eg. by pipetting. The sample is contained within the capture region 69 by the second switchable barrier 70. The enzyme conjugate is solubilised and if the analyte of interest is present in the sample it becomes bound to the immobilised antibody and the enzyme conjugate. The bottom edge 63 of the membrane is then placed in water (or other suitable diluent). Water flows along the strip 61

around the channel 66 but cannot flow into the channel 66 from below because of the first barrier 64. Flow continues along the strip 61 towards the rectangular line of surfactant 71. When surfactant reaches the second barrier 70, the second barrier 70 is switched to allow water to flow therethrough. The capture region 69 is than totally open to flow and sample and any excess enzyme conjugate is washed upwards away from the capture region 69.

During this time flow enters the channel 66 from above and the colorimetric reagent and line of surfactant 68 move towards the first barrier 64. When the barrier 64 is switched, flow in the channel 66 is reversed and the colorimetric reagent moves over the capture region 69. If the analyte of interest is present, the colorimetric substrate reacts with the enzyme conjugate to produce visible colour at the capture region 69. In this embodiment, the relative dimensions of the device and positioning of the various elements of the device are such that the device becomes totally saturated at substantially the same time as the colorimetric reagent reaches the capture region, so that the colorimetric reagent remains at the capture region.

The device is also designed so that the first barrier 64 is switched after the second barrier 70 to ensure that the colorimetric reagent passes into the capture region 69 and not around it.

This embodiment provide a means of analysing sample using a multi-reagent assay. It will be noted that excess conjugate is automatically washed away from the capture region 69 prior to arrival of colorimetric

reagent at the capture region 69. The device is particularly suited for screening assays and offers an automatic non-instrumental alternative to microtitration plate assays.

Example 5 describes a device having a single capture region 69, but in a modification, several capture regions are provided so that a number of samples can be analysed in a single assay.

Example 6

Referring to Figure 6a, a rectangular 12 µm nominal pore size unsupported nitrocellulose membrane strip 80 (type AE 100, Schleicher & Schuell, Dassel, Germany) is marked with impervious pen lines 81 of neat Lumocolor ink parallel to and marginally spaced from its long side edges 82. Parallel to a first short side edge 83, a first Type 2 switchable barrier 84 extends partly across the strip 80 (plotted using a 0.7 mm pen at 1 cm/s and dried at 35° C for 10 minutes). An impervious line 85 extends perpendicularly from each end of the first barrier 84 away from the first side edge 83 towards a second short side edge 86 to define a channel 87 having a closed first end and an open second end. Within the channel 87 is a gold conjugate containing region 88 spaced to the left of a line of surfactant 89 (10% ODG plotted using a 0.7 pen at 5 cm/s, and dried at 35° C for 10 minutes). Disposed between the second short side edge 86 and the channel 87, a capture region 90 is defined within an elliptical ring of a second Type 2 switchable barrier 91. The capture region 90 contains immobilised antibody. An elliptical ring of surfactant 92 (also 10% ODG) surrounds the capture region 90 and is spaced from the second barrier 91.

Referring to Figure 6b, the unsupported membrane strip 80 of Figure 6a is adhered to a polyester backing 93 (0.025 mm thick type 7759 film coated on one side with acrylic adhesive AS-110, Adhesives Research Ireland Ltd, Limerick, Ireland) and mounted to a housing 94. It should be noted that for unsupported membrane material, one face tends to have larger pores than the other face. Under low liquid pressure loads, liquid does not flow easily through the membrane from the large pore face to the small pore face. The polyester backing 93 is adhered to the large pore face of the membrane strip 80 (identified by being less shiny than the small pore face). The backing 93 is provided with an aperture 95 therethrough which is aligned with the capture region 90 of the membrane strip 80.

The housing 94 is machined from Perspex acrylic sheet (ICI, Darwen, UK) and is generally cuboidal. First and second troughs 96,97 extend partly across the width of the housing 94. The second trough 97 is deeper than the first trough 96 and is in communication therewith by virtue of a flow passage 98. The base of the flow passage 98 is higher than the base of the first trough 96 so that a lip 99 is defined between the first and second troughs 96,97. Within the first trough 96 is embedded a peg 100 which projects vertically. The polyester backed membrane is mounted flat on an upper surface of the housing 94 in such a position that the capture region 90 is directly above the peg 100 in the first trough 96. An end portion of the membrane is bent downwardly so that its first short side edge 83 is in contact with the base of the second trough 97.

In use, sample is applied to the capture region 90 using a pipette (in an alternative embodiment, not shown, a funnel is positioned above the

capture region 90 and loaded with a pre-determined volume of sample which is automatically applied to the membrane at a desired rate) and is constrained from flowing into the surrounding membrane by the second barrier 91. Sample quickly flows vertically through the thickness of the membrane 80 from the small pore face of the membrane 80 to the large pore face and through the aperture 95 in the polyester film 93. If the analyte of interest is present it will become bound to the immobilised antibody capture region 90. Pin 100 helps to guide flow down into the first trough 96. When sufficient sample is applied, sample flows over the lip 99 into the second trough 97 and wets the first short side edge 83 of the membrane 80. Sample flow is then as described with reference to Example 5 resulting in (i): washing of unbound sample from the capture region 90, (ii) flow of gold conjugate over the capture region 90 and (iii) flow of excess gold conjugate away from the capture region 90.

This embodiment allows a large volume of sample to be analysed, important if the analyte of interest is present in low concentrations. It is particularly suited for detecting bacteria (eg. Coliforme) in diluted samples as is often required in environmental tests.

In a modification of the above embodiment, the troughs 96,97 contain porous or absorbent material to absorb excess liquid and prevent it from escaping from the housing 94 after use. It will be understood that the purpose of the troughs 96,97 in the housing 94 is to enable a predetermined volume of sample to be applied to the capture region 90 before flow is initiated along the membrane 80. The same effect may be achieved by other methods. For example, in another modification (also

not shown), sample flowing through the membrane passes into porous material, or a capillary channel which is in abutment with the first short edge of the membrane. Suitable porous materials include glass fibre fleece, cellulose fibre fleece, polymeric sponge-like material and poly(isobutylene-co-maleic acid) sodium salt, with good absorbent properties.

FIGURE 1

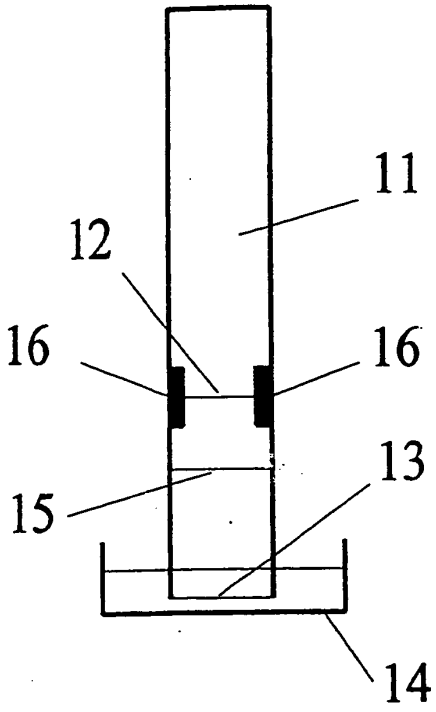
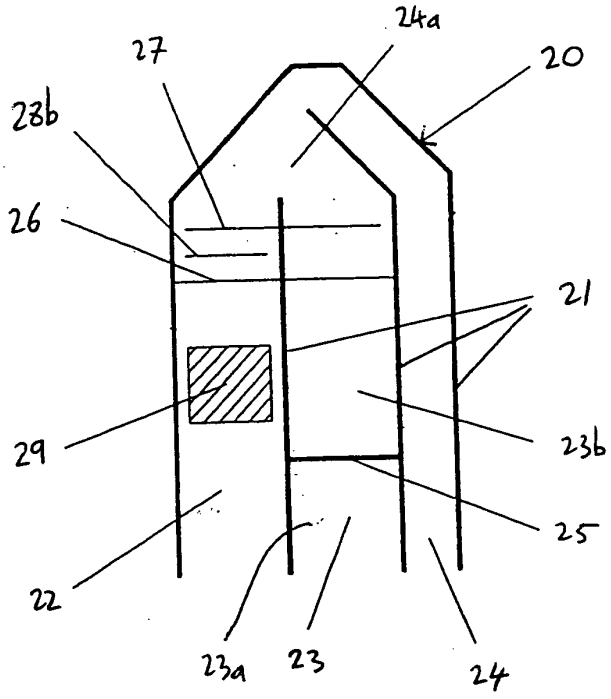


FIGURE 2b



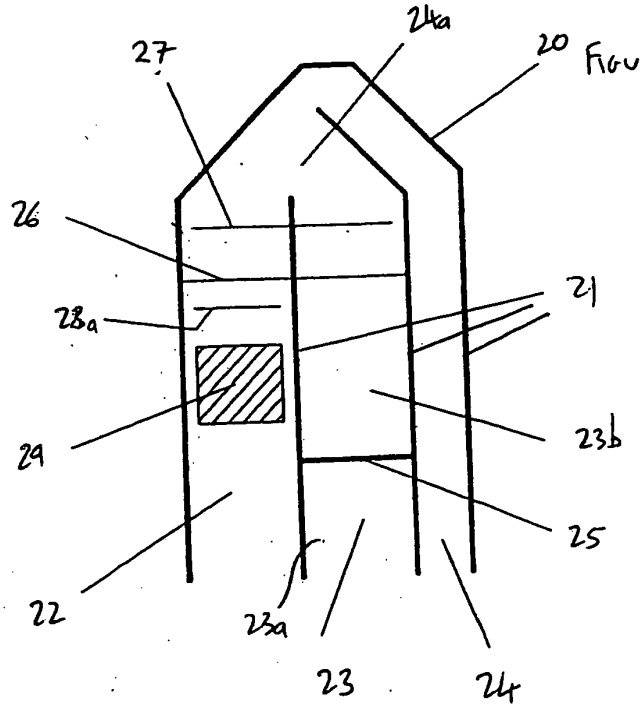


FIGURE 2a

FIGURE 3

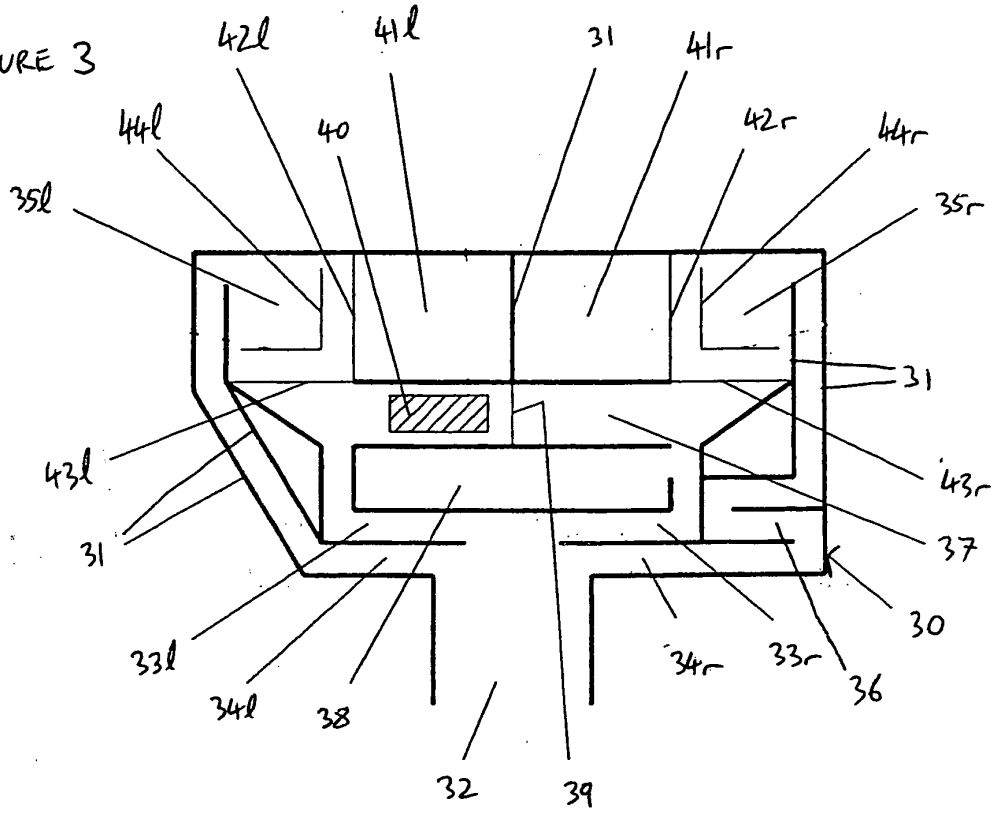


FIGURE 4

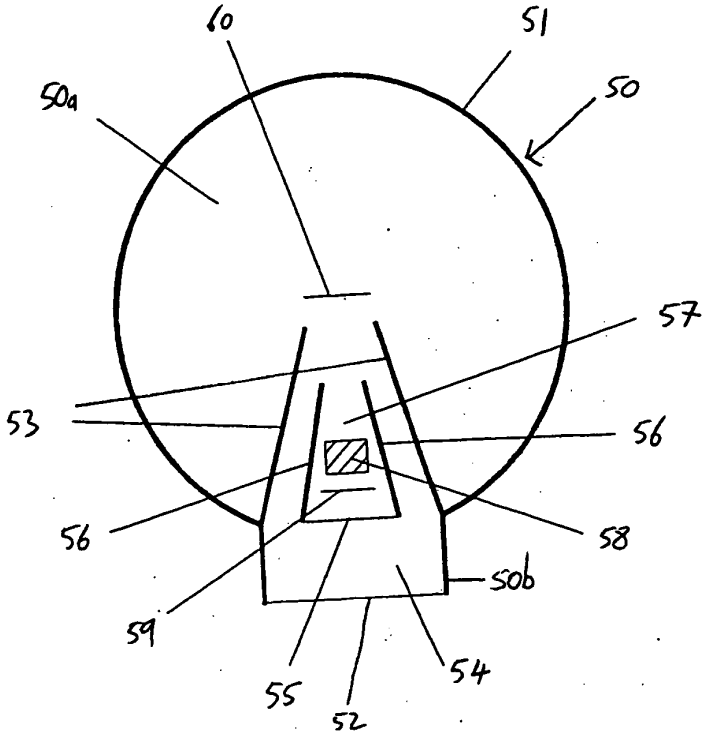


FIGURE 5

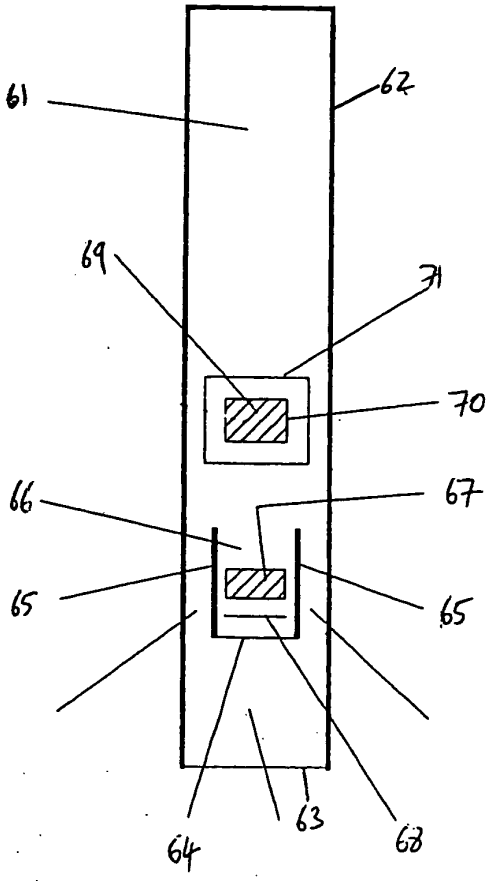


FIGURE 6a

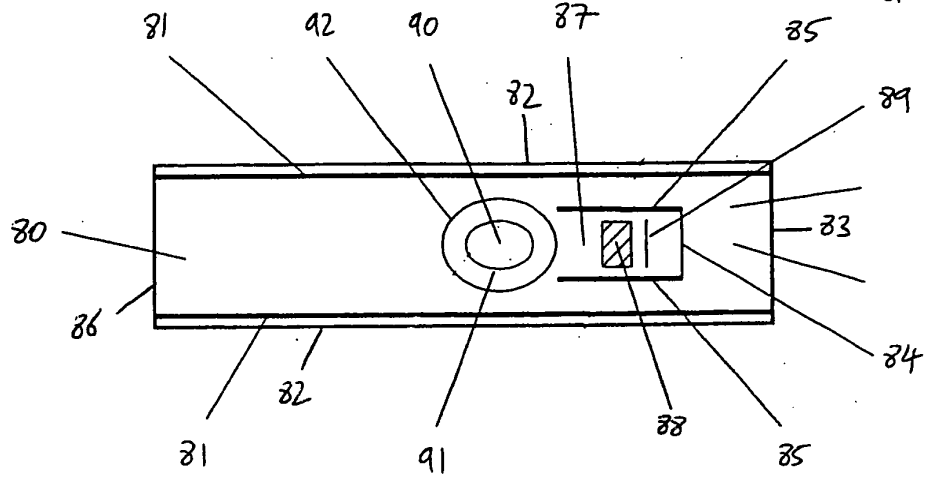
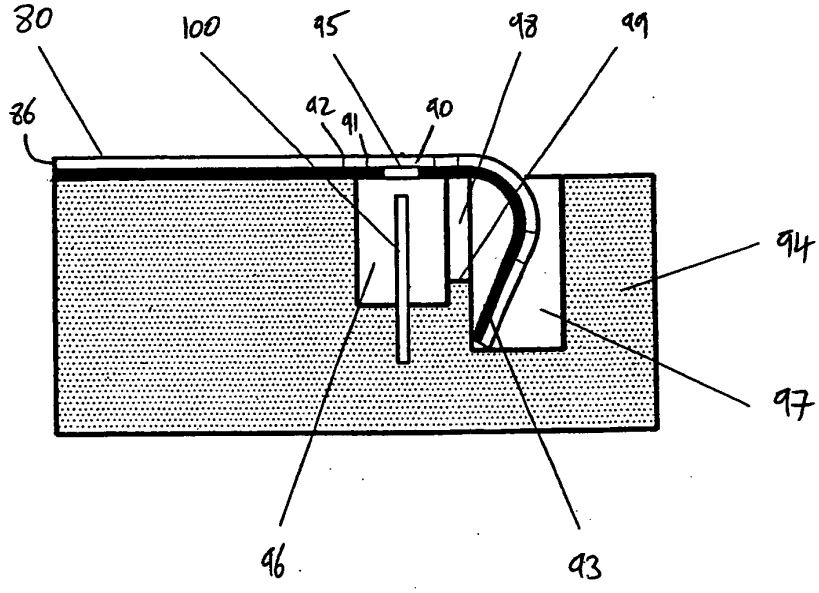


FIGURE 6b



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